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Monoamine Oxidase Inhibition Cannot Account for Changes in Visual Evoked Potentials Produced by Chlordimeform

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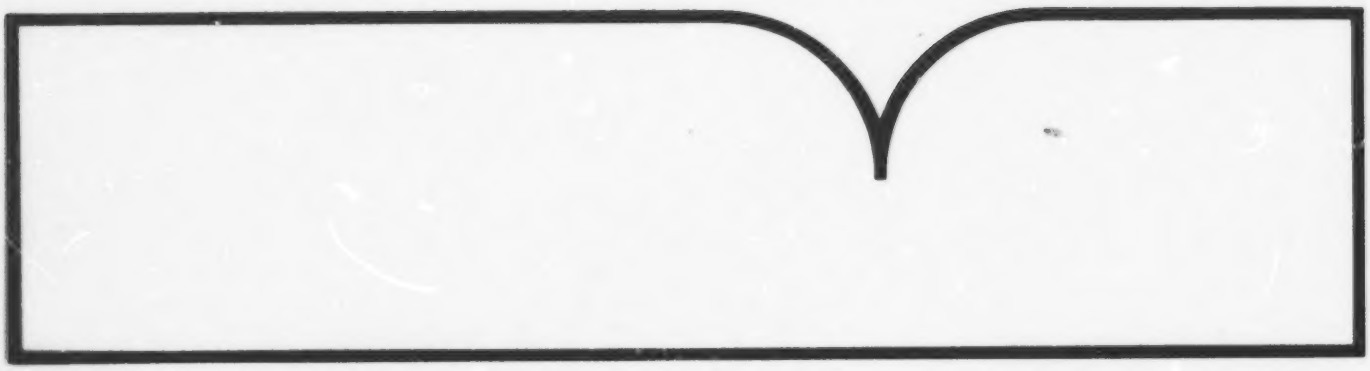

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16. ABSTRACT

Chlordimeform (CDM), a formamidine insecticide and monoamine oxidase (MAO) inhibitor, has recently been shown to produce profound changes in visual evoked potentials of hooded rats (Dyer and Boyes, The Toxicologist, 3: 13, 1983). Two experiments were performed to determine if the evoked potential changes were a result of MAO inhibition. In the first, the degree of MAO inhibition in the brains of rats treated with CDM (1.0 to 100 mg/kg i.p.) was compared with that produced by MAO inhibitor pargyline (0.3.to 30 mg/kg i.p.). Both compounds preferentially inhibited MAO-B, although MAO-A was substantially inhibited at higher dosages. Pargyline was a relatively more potent MAO inhibitor than CDM, but not more efficacious. In the second study, pattern reversal evoked potentials (PREPs) and flash evoked potentials (FEPs) were recorded from groups of rats after treatment with either saline, 0.4 mg/kg pargyline, 20 mg/kg pargyline, or 40 mg/kg CDM. The latter two groups were selected so as to have similar levels of MAO inhibition, about 90% inhibition of MAO-B and 60% inhibition MAO-A. The results showed a doubling of PREP amplitude and increased PREP and FEP latencies in the CDM-treated group, but no significant changes from saline control values in the pargyline-treated groups. These results confirm that CDM is an MAO inhibitor at dosages which produce behavioral and electrophysiological changes, but demonstrate further that the visual evoked potential changes produced by CDM are not a direct result of MAO inhibition.

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MONOAMINE OXIDASE INHIBITION CANNOT ACCOUNT FOR CHANGES IN VISUAL EVOKED POTENTIALS PRODUCED BY CHLORDIMEFORM

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Summary—Chlordimeform (CDM), a formamidine insecticide and monoamine oxidase (MAO) inhibitor, has recently been shown to produce large changes in visual evoked potentials of hooded rats (Boyes and Dyer, 1984a). Two experiments were performed to determine if the changes in evoked potentials were a result of the inhibition of MAO. In the first, the degree of inhibition of MAO in the brains of rats treated with chlordimeform (1.0–100 mg/kg, i.p.) was compared with that produced by pargyline (0.3–30 mg/kg, i.p.). Both compounds preferentially inhibited MAO-B, although MAO-A was substantially inhibited at larger doses. Pargyline was a relatively more potent inhibitor of MAO than chlordimeform, but not more efficacious. In the second experiment, pattern reversal evoked potentials (PREPs) and flash-evoked potentials (FEPs) were recorded from groups of rats after treatment with either saline, 0.4 mg/kg pargyline, 20 mg/kg pargyline or 40 mg/kg chlordimeform. The latter two groups were selected so as to have similar levels of inhibition of MAO, about 90% inhibition of MAO-B and 60% inhibition of MAO-A. The results showed a doubling of the amplitude of pattern reversal evoked potentials and increased latencies of the pattern reversal evoked potential and the flash-evoked-potentials in the chlordimeform-treated group, but no significant changes from saline control values in the pargyline-treated groups. These results confirm that chlordimeform is a MAO inhibitor at doses which produce behavioral and electrophysiological changes, but demonstrate further that the changes in visual evoked potentials produced by chlordimeform are not a direct result of the inhibition of MAO.

Key words: chlordimeform, formamidine pesticide, pattern reversal evoked potentials, flash evoked potentials, visual evoked potentials, monoamine oxidase inhibition, pargyline, rats.

Chlordimeform (CDM) is the prototype member of the formamidine class of insecticide/acaricide. The unique spectrum of biological actions produced by formamidines has been recently reviewed (Hollingworth and Lund, 1982; Knowles, 1982; Matsumura and Beeman, 1982). Although the mechanism of intoxication is unknown, the formamidines are somewhat unusual among pesticides in that they do not act on cholinergic neurotransmitter systems. Instead, chlordimeform and other formamidines have generated interest in part because of their actions on monoaminergic neurotransmitter systems. In mammals, the initial indications of a monoaminergic action came from reports that chlordimeform and its *N*-demethylated metabolite inhibited monoamine oxidase in rat liver (MAO) *in vitro* (Beeman and Matsumura, 1973; Aziz and Knowles, 1973). These reports raised the possibility that the intoxication with chlordimeform was the result of increased synaptic concentrations of monoaminergic neurotransmitters. Subsequently, chlordimeform was shown to inhibit MAO in rat brain *in vitro* in a competitive and reversible manner (Benezet and Knowles, 1976). Further studies showed that chlordimeform inhibited MAO in rat brain *in vivo*, and increased levels of serotonin, norepinephrine and dopamine in brain (Matsumura and Beeman, 1976; Benezet, Chang and Knowles, 1978; Maitre, Felner, Waldmeier and Kehr, 1978). In addition, type B MAO was found to be more inhibited by chlordimeform than type A MAO (Maitre *et al.*, 1978; Bailey, Martin and Downer, 1982), although others have reported the reverse preference (Benezet and Knowles, 1976; Hollingworth, Leister and Ghali, 1979), or approximately equal preference (Kaloyanova, Bainova and Zaprianov, 1981). Unfortunately, assay procedures for MAO employed previously have measured inadequately the amount of enzyme inhibition produced by chlordimeform, since failure to account for the reversibility of the reaction will under-estimate the amount of inhibition of MAO actually produced *in vivo*.

There is considerable evidence questioning the role of the inhibition of MAO in the intoxication with chlordimeform. The lethality of chlordimeform was not reduced by treatment of rats with either noradrenergic or serotonergic blockers or depletors (Robinson, Smith, Zelenski and Endecott, 1975; Robinson and Smith, 1977) and lethality was not increased by an α-adrenergic agonist (Robinson and

Smith, 1977). In contrast to these findings, however, Pfister, Chinn, Noland and Yim (1978) enhanced the intoxication with chlordimeform with three compounds, pyribenzamine, phenoxybenzamine and imipramine, all interacting with noradrenergic functions. In other studies, the rapid onset of excitation and tremor in rats treated with chlordimeform did not resemble the symptomatology generally associated with the inhibition of MAO (Matsumura and Beeman, 1976). Further, chlordimeform and related compounds did not show a good correlation between the potency of inhibition of MAO and lethal potency (Neumann and Voss, 1977; Hollingworth *et al.*, 1979). Thus, although chlordimeform is an unequivocal inhibitor of MAO, the available evidence indicates that lethality and some signs of intoxication may not be a consequence of the inhibition of MAO. Whether the inhibition of MAO can account for other, more subtle actions of chlordimeform is at present unknown.

It has recently been reported that acute exposure to chlordimeform produced large, selective changes in the visual evoked potentials of hooded rats (Boyes and Dyer, 1984a). The amplitude of pattern reversal evoked potentials (PREPs) was increased by chlordimeform to 200–300% of control values, yet the amplitude of flash-evoked potentials (FEPs) in the same rats was unchanged. Recent evidence indicates that the increase of the amplitudes of pattern reversal evoked potentials is dependent on stimulus contrast, occurring only at high contrast values (Boyes and Dyer, 1984b). This could account for the failure of chlordimeform to increase the amplitudes of flash-evoked potentials, the strobe-flash stimulus being devoid of contrast.

Discovery of the mechanism of action through which chlordimeform alters visual function could be of interest to both visual scientists, and to those interested in the actions of pesticidal compounds on the function of the mammalian nervous system. The selective, yet reversible nature of the effect of chlordimeform suggested the disruption of specific neurotransmitter systems as one possible mode of action. Visual function is dependent on monoaminergic neurotransmission (Bonting, 1976) and monoaminergic disruptions can alter visual evoked potentials in the rat (Dyer, Howell and MacPhail, 1981; Onofrj and Bodis-Wollner, 1982). Unfortunately, studies of the effects of MAO inhibitors on sensory-evoked potentials have generally used human subjects receiving the inhibitors for therapeutic reasons, in which case confounding factors make direct assessment of the consequences of the inhibition of MAO difficult. Reports of the effects of inhibitors of MAO on the evoked potentials of laboratory animals have not to date employed pattern-reversal stimuli. However, acute treatment with MAO inhibitors can produce rapid changes in animal behavior and auditory evoked potentials (e.g. Pellet, Weiss and Gourdon, 1983). The prominence of reports of the inhibition of MAO produced by chlordimeform, and the importance of monoaminergic systems in visual function, raised the possibility that the changes produced by chlordimeform in visual evoked potentials in the rat could be a consequence of the inhibition of MAO.

To ascertain if changes in visual evoked potentials in rat induced by chlordimeform resulted from the inhibition of MAO, the changes in evoked potentials and the inhibition of MAO produced by chlordimeform were measured and compared with those produced by a prototype inhibitor of MAO, pargyline. The assay of MAO was specifically designed to account for the reversible nature of chlordimeform induced inhibition of MAO. The results demonstrated that at equivalent doses for the inhibition of MAO in rat brain, treatment with pargyline failed to produce changes in visual evoked potentials similar to those of chlordimeform. Thus, changes induced by chlordimeform in visual evoked potentials in rat do not result solely from the inhibition of MAO.

METHODS

Activity of MAO

Adult male Long–Evans rats (Charles River, 275–325 g) were injected intraperitoneally with either pargyline HCl (Sigma Chemical Company; 0.3–30 mg/kg), chlordimeform HCl (NOR-AM Agricultural Products, Naperville, Illinois, 97% purity; 1–100 mg/kg), or the isotonic saline vehicle (1 ml/kg). Five subjects received each dose. Two hours after the injection the rats were decapitated, and the brains were rapidly removed, homogenized using a Teflon/glass system and kept on ice. The brains of the rats treated with pargyline were diluted in 10 vol of 0.32 M sucrose/50 mM sodium phosphate buffer (pH 7.4) followed by differential centrifugation at 4°C to produce a crude mitochondrial pellet (P2 pellet; Mailman and Morell, 1982) which was resuspended in 50 mM sodium phosphate buffer (pH 7.4). The activity of MAO was assessed by a radiometric procedure similar to that described by White and Wu (1975). [^{14}C]Serotonin binoxalate (5-HT; 50.7 mCi/nmol, New England Nuclear Co.; 0.2 μCi in a final substrate concentration of 0.2 mM) and [^{14}C]β-phenylethylamine HCl (PEA; 50.0 mCi/nmol, New England Nuclear Co.; 0.2 μCi in a final substrate concentration of 10 μM), were used as specific substrates of types A and B MAO, respectively (Suzuki, Katsumata, Oya and Matsumoto, 1979; Yang and Neff, 1973). Tissue, buffer and substrate (0.1 ml of each) were incubated together for 20 min at 37°C and then the reaction was stopped with 0.2 ml 2 N HCl. The deaminated metabolites were extracted into toluene–ethyl acetate (1:1) and counted by liquid scintillation spectrometry (Beckman LS-7000). The degree of quench, calculated using an external standard, was equivalent in all samples. Counting efficiency for the ^{14}C-channel was about 55%. Blank

samples contained either buffer instead of tissue, or 0.67 mM pargyline to inhibit all activity of MAO. Protein content of each sample was determined by the method of Lowry, Rosebrough, Farr and Randall (1951) using bovine serum albumin as standard. Data were calculated as counts (cpm) per mg protein and expressed as percentage inhibition relative to values of the saline control.

A procedural modification was necessary to assay the inhibition of MAO produced by chlordimeform, since chlordimeform is a reversible inhibitor (Benezet and Knowles, 1976; Hollingworth *et al.*, 1979). To minimize dilution of the insecticide at the active site, brains of rats treated with chlordimeform were homogenized in 2 vol of 0.32 M sucrose/50 mM sodium phosphate buffer, pH 7.4 (H. White, personal communication). Preliminary experiments confirmed that the measured amount of inhibition of MAO decreased with increasing dilution of the tissue. A reaction volume of 0.15 ml (0.1 ml tissue, 0.05 ml substrate) was incubated for 15 sec with [^{14}C]phenylethylamine or for 60 sec with [^{14}C]5-hydroxytryptamine, during which times the rate of production of deaminated metabolites was linear. The remainder of the assay and analysis were as described above.

Electrophysiology

For evoked potential studies, the general experimental procedures were identical to those described elsewhere (Dyer *et al.*, 1981; Boyes and Dyer, 1983). Briefly, adult male Long–Evans hooded rats (Charles River) weighing 250–400 g were used. Chronic epidural stainless-steel electrodes were surgically implanted (0.35 ml/100 g Chloropent anesthesia, Fort Dodge Lab. Inc., Fort Dodge, Indiana) at least 1 week prior to the experiment. For each rat, the active recording electrode was placed over visual cortex 6 mm posterior to bregma and 4 mm lateral to midline, and reference and ground electrodes were placed 2 mm anterior to bregma and 2 mm to the right and left of midline, respectively.

Pattern reversal evoked potentials were recorded from harness-restrained, awake rats placed in front of the TV screen of a Grass Model VPG pattern generator. The eliciting stimulus of the pattern reversal evoked potentials was a black-and-white square-wave grating (99% contrast, 30 cd/m^2). Evoked responses were recorded after every pattern shift, which occurred at a rate of 0.5 Hz and averaged over 200 trials. Flash-evoked potentials were recorded in a small mirrored chamber with the strobe lamp of a Grass model PS-22 photostimulator on one wall. The stimulator was set at an intensity of 16, which provided a 10-μsec flash of approx. 4.53×10^7 lx peak power. Flash stimuli were presented in pairs with a 200-msec inter-stimulus interval to provide a measure of recovery. Pairs of flashes were presented at 0.5 Hz and 128 trials were averaged.

For evoked potential studies, treatment groups received intraperitoneally, either 0.4 mg/kg pargyline HCl, 20 mg/kg pargyline HCl, 40 mg/kg chlordimeform HCl (all doses expressed as the total salt), or isotonic saline (1 ml/kg) as the vehicle control ($n = 16$ for each group). Testing began 2 hr after injection and lasted approx. 30 min. Three averaged evoked potentials were recorded from each rat: a pattern reversal evoked potential, a flash-evoked potential and a brainstem auditory evoked response elicited by a click (BAER). Test order was counterbalanced between the rats. The brainstem auditory evoked response data have been presented elsewhere (Janssen, Boyes and Dyer, 1983).

Statistical analysis of evoked potential data followed the guidelines of Muller, Otto and Benignus (1983).

RESULTS

On visual observation, the rats treated with chlordimeform at doses of 30 mg/kg and more showed a dose-related increased excitability and myotonic tremors. Periods of listlessness were observed after the administration of 100 mg/kg chlordimeform and 30 mg/kg pargyline. The rats receiving smaller doses of pargyline were not visibly different from controls. Three rats receiving 100 mg/kg chlordimeform had spontaneous tonic seizures and died. One of the deaths occurred immediately prior to sacrifice, whereas the other two occurred before the 2-hr time period had elapsed. Data from the latter 2 rats were not used.

The inhibitory properties of pargyline and chlordimeform on MAO are shown in Fig. 1. For both compounds, MAO-B appeared to be more sensitive than MAO-A as shown by the greater suppression of the metabolism of phenylethylamine than 5-HT at

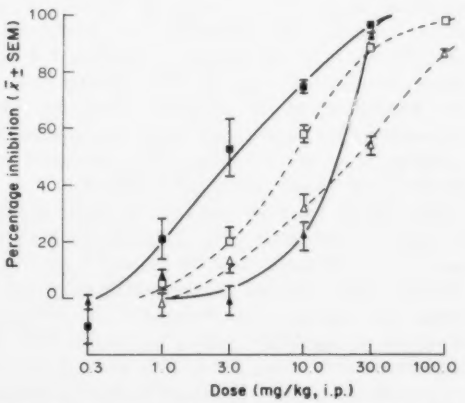

Fig. 1. Inhibition of MAO produced by pargyline (solid symbols) and chlordimeform (CDM; open symbols), expressed as percentage inhibition (mean ± SEM). The metabolism of phenylethylamine is indicated by squares, and the metabolism of 5-HT by triangles. The sample size for all points is 5 except at chlordimeform 100 mg/kg where $n = 3$. Curves were fitted by eye.

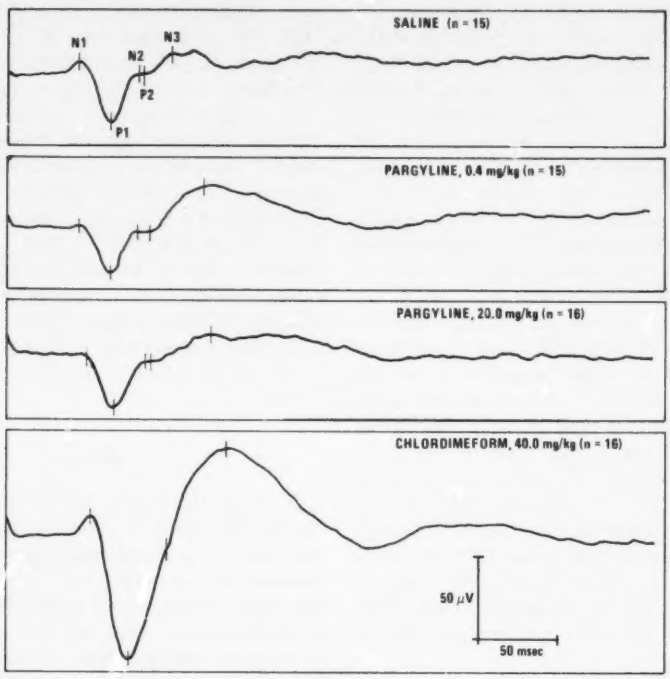

Fig. 2. Pattern reversal evoked potential average waveforms from rats treated with either saline, pargyline or chlordimeform (CDM). The pattern reversal evoked potential was recorded from a visual cortex electrode following the abrupt reversal of black-and-white grating, and averaged over 200 trials. The waveforms presented are group averages constructed from the averaged evoked potentials of individual rats in order to show group trends. Pargyline produced no significant changes in pattern reversal evoked potentials. Chlordimeform increased pattern reversal evoked potential peak-to-peak amplitudes, made peaks N2 and P2 undetectable and increased the latency of peak P1.

each dose. This relative selectivity for MAO-B at small doses of pargyline has been reported previously (Fuentes and Neff, 1975). In contrast, chlordimeform exhibited a greater separation of effects at large doses. Pargyline was somewhat more potent than chlordimeform as an inhibitor of MAO, but not more efficacious. For the evoked potential studies, the selected doses of pargyline (20 mg/kg) and chlordimeform (40 mg/kg) were approximately equieffective, each producing 90% inhibition of MAO-B and 60–64% inhibition of MAO-A.

The group average pattern reversal evoked potential of control rats (Fig. 2) was similar in shape and size to previous results (Boyes and Dyer, 1983, 1984a). Pattern reversal evoked potentials recorded from rats treated with either 0.4 or 20 mg/kg pargyline were generally similar to controls, with all the components being identifiable and of normal amplitude and latency. The slight increase in latency seen in some components of the waveforms did not reach statistical significance. In contrast, the average pattern reversal evoked potential from rats treated with chlordimeform showed a large increase in both peak amplitudes and latencies.

The group mean amplitudes and latencies of pattern reversal evoked potentials for the four dose groups are presented in Fig. 3. Because components N2 and P2 could not be reliably identified in the waveforms of rats treated with chlordimeform only the data for peaks N1, P1 and N3 are reported. Both the peak-to-peak amplitudes of components N1P1 and P1N3 of the group treated with chlordimeform were over twice the values of the other groups, whereas the corresponding values of the groups treated with pargyline were not substantially different from saline-treated controls. Statistical analysis (MANOVA) of the peak-to-peak amplitudes of pattern-reversal evoked potentials revealed a significant overall effect of treatment [$F(6,108) = 10.66$, $P < 0.0001$]. Stepdown analysis of variance revealed significant effects of treatment for both N1P1 and P1N3 [both $F(3,56) > 16.67$, both $P < 0.0001$]. Significant changes from control amplitudes were solely attributable to the chlordimeform-treated group (Duncan's test, $P < 0.0001$).

The peak latencies of pattern reversal evoked potentials were greater in the group treated with chlordimeform than in the others, but only the latency of the P1 component was substantially increased (Fig. 3). Analysis of the data on the latency

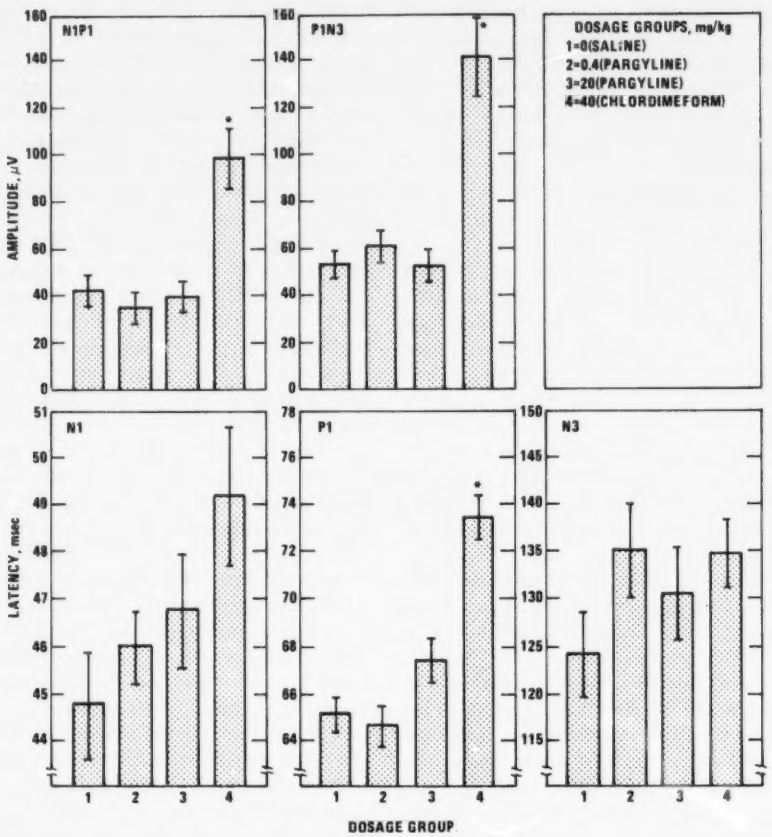

Fig. 3. Pattern reversal evoked potential peak-to-peak amplitudes and peak latencies as a function of dose. Plotted values represent mean ± SEM. Statistically significant changes are indicated with an asterisk.

of pattern reversal evoked potentials revealed a significant overall effect of treatment [MANOVA $F(9,158) = 10.61$, $P < 0.0001$], and a significant step-down analysis of variance effect of treatment for the peak latency of the P1 component [$F(3,56) = 24.75$, $P < 0.0001$] which was attributable to an increase in the group treated with chlordimeform (Duncan's test, $P < 0.0001$). The groups treated with pargyline were not significantly different from saline-treated control.

Regarding the data on flash-evoked potentials, none of the groups showed trends of treatment-related changes of peak amplitudes, but increases in peak latency did occur in the group treated with chlordimeform. The data on the amplitude of flash-evoked potentials were not included in the statistical analysis because previous experiments had shown the amplitudes of flash-evoked potentials to be unchanged by chlordimeform (Boyes and Dyer, 1984a). For the first of the paired flashes, the latencies of the N1 and P2 components were greater in the group treated with chlordimeform than in the others. Data analysis yielded a statistically significant change in peak latency of flash-evoked potentials [MANOVA $F(18,161) = 4.96$, $P < 0.0001$], statistically significant analysis of variance for peaks N1 and P2 [both $F(3,60) > 8.93$, both $P < 0.0001$], but a nonsignificant analysis of variance for the other flash-evoked potential peaks. Duncan's test revealed that the group treated with chlordimeform was different from controls for the latency of peaks N1 (32.6 ± 0.6 vs 30.6 ± 0.4 msec) and P2 (51.9 ± 0.8 vs 45.6 ± 0.7 msec, respectively). Paired-flash recovery functions were not significantly altered by exposure to chlordimeform or pargyline [MANOVA $F(33,146) = 1.32$, $P > 0.1368$].

DISCUSSION

Although several groups of investigators have shown chlordimeform to be an inhibitor of MAO, both *in vitro* or *in vivo* (Benezet and Knowles, 1976; Hollingworth *et al.*, 1979; Maitre *et al.*, 1978), discrepancies exist concerning the magnitude and specificity of this effect. Previous *in vivo* studies used heavily diluted tissue fractions (1:10, 1:20; e.g. Bailey *et al.*, 1982; Benezet *et al.*, 1978; Maitre *et al.*, 1978), a procedure which underestimates the amount of

inhibition of enzyme produced *in situ* by a reversible inhibitor such as chlordimeform. The current procedures employed minimal tissue dilution, and as a consequence, MAO in rat brain was found to be inhibited at low dose levels of chlordimeform, and to a greater degree than reported previously. Enzyme activity was inhibited by 50% at about 8 mg/kg chlordimeform for MAO-B, and at about 30 mg/kg for MAO-A. The degree of inhibition progressed from slight to severe over the range of behaviorally-active doses (3–30 mg/kg; MacPhail and Leander, 1980, 1981; Pfister, Hollingworth and Yim, 1978; Witkin and Leander, 1982). Although the magnitude of the effect of chlordimeform was greater than previously obtained with greater dilutions of brain tissue, these data may still slightly underestimate the degree of inhibition present *in situ*. Even the 1:2 dilution employed may have washed some of the agent from the sites of interaction with the enzyme. Thus, particularly for sub-lethal actions, the dismissal of the inhibition of MAO as a possible mechanism for intoxication with chlordimeform may be premature.

The present results show that exposure to chlordimeform had two consequences; it altered visual evoked potentials and inhibited MAO. Although the amount of inhibition of MAO in rats receiving 40 mg/kg of chlordimeform was substantial (estimated to be about 90% inhibition of MAO-B and 60% inhibition of MAO-A), it does not appear that inhibition of MAO can account for the changes in visual evoked potentials produced by chlordimeform. The amplitudes of pattern-reversal evoked potentials of rats receiving chlordimeform were twice those of rats receiving 20 mg/kg pargyline, even though the treatment with pargyline produced a similar degree of inhibition of MAO. In addition, chlordimeform increased the latency of pattern reversal evoked potentials and flash-evoked potentials, but no such changes were seen after pargyline. The failure of pargyline to alter flash-evoked potentials concurs with the statement of Sabelli, Vazquez and Flavin (1975) that injection with pargyline alone did not alter the flash-evoked potentials of rabbits. These findings demonstrate that the changes in visual evoked potentials induced by chlordimeform are not a consequence of the inhibition of MAO *per se*.

The mechanisms of action of chlordimeform responsible for the selective, large enhancement of the amplitudes of pattern reversal evoked potentials but not flash-evoked potentials remains unknown. There are many reported actions of chlordimeform which could be responsible (for reviews see Hollingworth and Lund, 1982; Knowles, 1982; Matsumura and Beeman, 1982). In subsequent experiments, it was found that hypothermia resulting from an acute dose of chlordimeform contributes to the prolonged peak latencies, but that the amplitudes of pattern reversal evoked potentials were still two or three times greater than control values in rats treated with chlordimeform and protected from hypothermia (Boyes, Padilla and Dyer, 1985). Recent evidence suggests that the *N*-demethyl metabolites of chlordimeform act as octopamine agonists in insects and that this activity corresponds well with the pesticidal action in certain species (Evans and Gee, 1980; Hollingworth and Murdock, 1980). Invertebrate octopamine receptors are similar to mammalian α_2-adrenergic receptors (Evans, 1980). One possibility is that chlordimeform, or its metabolites, could act directly on adrenergic receptors in the visual system, as has been shown in rabbit vascular muscle (Robinson and Bittle, 1979). Because α_2-receptors are found in the retina (Bittiger, Heid and Wigger, 1980; Hadjiconstantinou, Cohen, Rubenstein and Neff, 1984), the lateral geniculate nucleus and the superficial layers of neocortex (Unnerstall, Kopajtic and Kuhar, 1984), there are numerous possible sites of action for chlordimeform or its metabolites along the primary visual pathway. In this regard, an intriguing case was reported by Schafer and McKean (1975) who presented the flash-evoked potentials and the pattern reversal evoked potentials of a "profoundly retarded" phenylketonuric adolescent under conditions of high and low concentrations of phenylalanine in blood (their Fig. 1). In the high level phenylalanine condition both pattern reversal evoked potential and flash-evoked potential waveforms showed increased latencies, and the pattern reversal evoked potential, but not the flash-evoked potential, showed markedly increased amplitudes. The similarity of changes in evoked potentials coupled with possible monoaminergic disruption suggests that chlordimeform and large concentrations of phenylalanine in blood in the phenylketonuric patient may act through similar mechanisms.

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